ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN TG, LEP AND TFAM GENES WITH CARCASS TRAITS IN CROSS-BREED CATTLE

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ABSTRACT

The objective of this preliminary study is to determine the genotypes of genes using for Marker Assisted Selection for meat quality and to asses the association of single nucleotide polymorphisms in these genes in cross-breed *Bos taurus* cattle. For this study the previously reported polymorhpisms in genes thyroglobulin (*TG*), leptin (*LEP*) and mitochondrial trascription factor A (*TFAM*) were chosen due to their possible association with marbling and carcass traits in cattle.

Analysed carcass traits were following: kidney and pelvic fat, netto gain, weight of tenderloin and weight of rib eye. A crossbred population of 109 animals (Czech Spotted Cattle, Holstein, Red Holstein, Ayshire) was developped in Research Institute for Cattle Breeding, Ltd. In Rapotín. The analysed polymorphisms were determined by PCR-RFLP.

Present study shows no significant associations of the SNPs in *TG* and *TFAM* genes for any traits, on the other hand significant association between genotype *C/T* in exon 2 of *LEP* gene and deposition of kidney and pelvic fat ($P<0,05$) was observed. This result suggests that allele *T* is responsible for higher fat deposition. Other association of SNP in *LEP* gene shows significant difference between genotype *CC* a *CT* and netto gain ($P<0,05$) and high significant difference between genotype *CC* and *CT* ($P<0,01$) – it would mean that heterozygous genotype is undesirable. Our results can be influenced by low number of tested animals or by unequal genotype distribution in selected population (*LEP* gene) and by analysed traits; there were only the information from slaughter about carcass.

The obtained results suggest possible using these genes in next part of project because of their significant association (*LEP* gene) or their previously presented associations (*TG* and *TFAM* gene). It is possible that it will be found a significant association between genotypes of these genes and marbling (intramuscular fat content) and other meat quality characteristics which will be analysed in the next part of our project with higher number of animals.

Key words: *TG, LEP, TFAM,* meat quality, cattle

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INTRODUCTION

Both genetic and environmental factors are known to influence beef quality and carcass traits and variation in several candidate genes has been shown to be associated with carcass composition, e.g. thyroglobulin (gene symbol *TG*), leptin (gene symbol *LEP*) or mitochondrial transcription factor A (gene symbol *TFAM*) (Jiang *et al*., 2005; Gutiérrez-Gil *et al*., 2008).

Candidate gene proposed to affect marbling and other carcass trait produces thyroglobulin, the precursor to thyroid hormones with known endocrine roles in fat metabolism (Barendse, 1999; Casas et al., 2007). Thyroid hormones triiodothyronine (T3) and thyroxine (T4) play important role in regulating metabolism and can affect adipocyte differentation and growth (Gan et al., 2008). The TG is considered as a functional and positional candidate gene for QTL with an effect in fat deposition (Thaller et al., 2003; Gan et al., 2008). The TG gene has been mapped to the centromeric region of bovine chromosome 14. In our study we have chosen the previously reported SNP C/T in repetitive element upstream from promoter of the TG gene located at position 422 of accession no. X05380 (Barendse, 1999). This marker TG5 has been put into commercial DNA test for marbling in USA.

Leptin is a 16-kDa protein product of the obese gene synthesised and secreted by adipocytes and its expression is regulated by body fatness, energetic balance, insulin or glucocorticoids (Passos *et al.*, 2007). The main leptin functions lies in informing the central nervous system about the size of fat storages and controlling feed intake, energy expenditure and whole-body energy balance; therefore, leptin concentration in plasma decreases in negative energy balance and feed intake increases (Kononoff *et al*., 2005; Passos *et al*., 2007). *LEP* gene has been mapped to bovine chromosome 4 and polymorphisms in the coding region of bovine *LEP* gene have been associated with serum leptin concentration, feed intake, milk yield and body fatness (Nkrumah *et al*., 2005). For our study a *C/T* transition that encoded an amino acid change of an arginine to a cysteine (Arg25Cys) indentified in exon 2 in *LEP* gene was chosen.

Mitochondrial transcription factor A is a nucleus-coded protein which regulates the initiation of transcription and replication of mitochondrial DNA and decreasing expression level of *TFAM* gene has been associated with onset of obesity in mice (Jiang *et al.*, 2005). Jiang *et al.* (2005) hypothesized genetic variants of this gene influence mitochondrial biogenesis consequently affecting body fat deposition and energy metabolism. The *TFAM* gene has been localised on bovine chromosome 28. Two SNPs (*A/C* and *C/T*) have been detected in promoter region in bovine *TFAM* gene and their statistic analysis suggests the association with marbling and body fat deposition in F_2 cross-breed Limousine and Wagyu population. For our study only the polymorphism *A/C* has been chosen because of close linkage between these 2 SNPs.

MATERIALS AND METHODS

Animals

A cross-breed population of 109 animals (Czech Spotted Cattle, Holstein, Red Holstein, Ayshire) was developped in Research Institute for Cattle Breeding, Ltd. In Rapotín.

Genotypes detection

DNA samples were extracted from meat samples by JETQUICK Tissue DNA Spin Kit (Genomed) and stored at -20°C until genotyping.

The PCR-RFLP method was used to detect the genotypes in *TG*, *LEP* and *TFAM* genes. For determination of allele *C* and *T* of the *TG* gene, a 548 bp fragment was amplified and digested with *Psu*I as described by Thaller *et al.* (2003). The allele *C* and *T* of *LEP* gene was distinguished by restriction enzyme *Kpn*2I with restriction site in 94 bp fragment as described by Buchanan *et al*. (2002). The 801 bp PCR fragment of *TFAM* gene was cut by restriction enzyme *Hae*III to distiguish allele *A* and *C* as described by Jiang *et al.* (2005). All PCR conditions were adapted for specifications of our laboratory (e.g. MJ Research PTC200 Thermo Cycler etc.). After digestion of PCR products the DNA fragments were separated on 2-3% agarose gels in electrophoresis visualised by ethidium bromide.

Analysed traits

The following traits were analysed: kidney and pelvic fat deposition (kg), netto gain (g), weight of tenderloin (kg) and weight of rib eye (kg).

Statistical analysis

The statistical analysis was performed by the general linear model (GLM) by SAS for Windows 9.1.4. The genotypes of relevant genes (G_{i-k}), SEUROP classification (SEU_l) and fat classification ($SEUtuk_m$) were used as fixed effects.

$$y_{ijklm} = \mu + G_i + G_j + G_k + SEU_l + SEUtuk_m + e_{ijklm}$$

RESULTS AND DISCUSSION

First objective of our study was to determine the genotypes of chosen markers in cross-breed population. All 3 markers were polymorphic and all 3 genotypes of each marker were detected. Genotype distribution and allelic frequency of relevant genes in analysed population are shown in Table 1. Observed frequencies of all 3 markers are in accordance with different authors (Thaller *et al.*, 2003; Jiang *et al*., 2005; Casas *et al.*, 2007).

Tab. 1 Number of animals with genotype of genes and allelic frequency of markers

Gene	Genotype count			Allelic frequency	
TG	CC	CT	TT	C	T
	62	43	4	0,77	0,23
LEP	CC	CT	TT	C	T
	81	20	8	0,83	0,17
TFAM	AA	AC	CC	A	C
	28	72	9	0,59	0,41

Marker TG5 in *TG* gene has been reported to be associated with fat content of some muscles, mainly musculus longissimus dorsi, in German Holstein (Thaller *et al*., 2003), with various marbling score (Barendse, 1999), with fat thickness and longissimus muscle area in *Bos indicus* cattle (Casas *et al.*, 2005) and in 3 different cross-breed population *TT* genotype had the numerically greatest marbling score (Casas *et al*., 2007). In our present study there was no significant association between genotype of TG5 marker in *TG* gene and any analysed traits.The least squares means and standard errors of all 3 genes are presented in Table 2.

SNP *C/T* in exon 2 of *LEP* gene has been reported to be associated with carcass fat level when allele *T* was associated with fatter carcass in unrelated bulls (Buchanan *et al*., 2002), *T* allele is associated with increasing carcass fat and the carcass weight of animals with *CC* genotype tended to be higher than of animals with *TT* genotype (Kononoff *et al.*, 2005). Present study shows significant association between genotype *C/T* in exon 2 of *LEP* gene and deposition of kidney and pelvic fat (P<0,05) when allele *T* is responsible for higher fat deposition; animals homozygous *TT* have the highest kidney and pelvic fat deposition. Other association of this SNP suggests significant difference between genotype *CC* a *CT* and netto gain and high significant difference between genotype *CC* and *CT* – it would mean that heterozygous genotype is undesirable because *CT* has the lowest netto gain and homozygous genotypes *CC* or *TT* has higher netto gain than *CT*. This result can be influenced by low number of animals or by unequal genotype ditsribution in selected population.

The polymorphism *A/C* in promoter of *TFAM* gene has been assiated with marbling and subcutaneous fat deposition (Jiang *et al*., 2005). Jiang *et al.* (2005) has reported *A/C* polymorphism in bovine *TFAM* gene that this SNP had the greatest effect on marbling and subcutaneous fat deposition among 4 candidate genes (*TG*, *DGAT1*, *LEP* and *TFAM*). Unfortunately in our preliminary study no significant association in *A/C* SNP were observed for any traits.

Tab. 2 Associations of analysed genes with 4 traits in cross-breed population

Marker	Analysed traits			
	DF	NG	WT	WRE
TG5				
CC	12,04±1,12	553,71±12,33	2,39±0,08	7,08±0,21
CT	12,39±1,25	556,65±13,82	2,30±0,09	6,86±0,23
TT	14,66±2,73	528,84±30,13	2,40±0,19	6,86±0,51
LEP				
CC	11,5±1,18*	556,16±13,01[b]	2,34±0,08	7,01±0,22
CT	11,79±1,70[a]	515,43±18,73*[b]	2,32±0,12	6,65±0,32
TT	15,80±2,13*[a]	567,61±23,45*	2,43±0,15	7,15±0,40
TFAM				
AA	12,81±1,50	550,45±16,57	2,43±0,10	6,97±0,28
AC	13,73±1,31	553,74±14,41	2,36±0,09	7,03±0,24
CC	12,54±2,09	535,02±22,99	2,30±0,15	6,81±0,39

*[a] significant difference beetween genotypes (P<0,05), [b] high significant difference between genotypes (P<0,01)

DF – kidney and pelvic fat deposition, NG – netto gain, WT – weight of tenderloin, WRE - weight of rib eye

CONCLUSION

This report is preliminary study of association of genes using for MAS with meat quality and carcass composition traits. The obtained results suggest possible using these genes in next part of project because of their significant association (*LEP* gene) or their previously presented associations (*TG* and *TFAM* gene). Our results can be influenced by low number of tested animals and by analysed traits; there were only the information from slaughter about carcass. It is possible that it will be significant association between genotypes of these genes and marbling (intramuscular fat content) and other meat quality characteristics which will be analysed in the next part of our project.

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